ON TEMPERATURE AND THE BREATHING RHYTHM OF CANIS MUSTELUS AND SQUALUS ACANTHIAS

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The casual observation of dogfishes in aquaria reveals immediately a noteworthy regularity in the rhythmic movement of the gill-slits. The movements of the spiracles are coördinated in a perfect way with those of the slits and they go on with the same regularity, without interruption, for long periods. All these features seemed to point to the suitability of this animal for a study of the frequency of gill movements as controlled by temperature.

The previous work of Bethe, Baglioni, and others had shown considerable irregularity in the Q_{10} ratio (*cf.* Kanitz, 1915); the observations of Crozier and Stier (1924–25) on the opercular breathing rhythm in the goldfish, on the contrary, showed regularity in this rhythm under properly controlled conditions and demonstrated the applicability of the Arrhenius equation to the data obtained. Would the dogfish obey the same rule and in a similar way?

For the observations, young specimens (25 to 37 cm. long) were kept in a large aquarium the temperature of which was maintained constant to within $\pm 0.05°$ C. for periods of 30 to 50 minutes at any given temperature. The number of points on the thermometric scale chosen for the observations was made as large as possible, and in general at each temperature from 20 to 30 series of 20 beats were timed with a stop watch. The regularity of the breathing rhythm at constant temperature is remarkable; countings of 20 to 30 successive series of 20 beats often agreed to within 1 to 2 per cent. The only exception seems to be around 15° C. where more erratic values are found. We will come back later to this point. Let us note also that Crozier and Stier have found agreement of the same order of magnitude. Care was taken to allow time for complete temperature equilibrium between the organism and the medium, the water of the aquarium also being stirred gently. The aquarium was almost completely insulated by a cover which cut out most of the light; a small portion of the surface only was left uncovered to permit observation. The water in the aquarium was kept as close as possible to saturation with air, so as to exclude any possible effect of O_2 concentration. Observations made

with waters having different concentrations of O_2 did not show any direct effect of oxygen concentration upon frequency of breathing movements, at least in the range considered. The first observations showed immediately the importance of having the animals perfectly quiet. Measurements can only be made if the animals have not been actively swimming for at least five minutes, the gill rhythm being speeded up after swimming. This is in harmony with the observations of Parker (1909) ". . . that the rate of gill movement in the dogfish depends upon the momentary state of movement of the animal. When resting they vary from 35 to 40 movements per minute. When swimming slowly they respire 50 to 55 times per minute. In vigorous swimming, the rate is doubtless still more rapid." These observations were made probably between 15° and 18° C., and the frequencies agree fairly well with those here found.

The first indications given by the observations show that the values for Q_{10} are rather erratic, as will be seen in Table I.

TABLE I

Q_{10} ratios

$$\frac{11.4° \text{ C.}}{21.5° \text{ C.}} = 2.06 \qquad \frac{12.70° \text{ C.}}{22.75° \text{ C.}} = 2.38 \qquad \frac{13.5° \text{ C.}}{23.5° \text{ C.}} = 1.72$$

$$\frac{14.5° \text{ C.}}{24.5° \text{ C.}} = 1.78 \qquad \frac{15.5° \text{ C.}}{20.5° \text{ C.}} = 1.21 \qquad \frac{17.5° \text{ C.}}{22.75° \text{ C.}} = 2.10$$

The data were treated to determine the goodness of fit of the Arrhenius equation (Fig. 1). The graph shows immediately that nearly all the points are distributed within three main bands, each with parallel edges; a fourth, small, short band parallel to one of the three main ones, contains the rest of the points. These bands may be called *I, Ia; II, III* (*cf.* Fig. 1). The values found for μ are:

$$I \text{ and } Ia \dots\dots\dots\dots\dots\dots 16,400$$
$$II \dots\dots\dots\dots\dots\dots\dots 8,200$$
$$III \dots\dots\dots\dots\dots\dots\dots 35,000$$

The first of these values corresponds exactly with that found for the opercular breathing rhythm of the goldfish by Crozier and Stier (1924–25). But, differing from what was found in the latter case, where only one temperature characteristic was secured, two additional values of μ appear (8,200 and 35,000). One should note the fact that the observational points which fall in *Ia,* were followed suddenly by points falling in the upper part of band *II,* without any smooth transition. The absolute values for the points in *Ia* are very different from those in *I,* but the slopes of both *I* and *Ia* are identical.

Another shift of points occurs also for *I*, where under certain not well-determined circumstances, in the neighborhood of 14.5° C., a sudden shift to *II* may be found. These cases of shift from one band to another are analogous to the ones found by Crozier and Stier (1925–
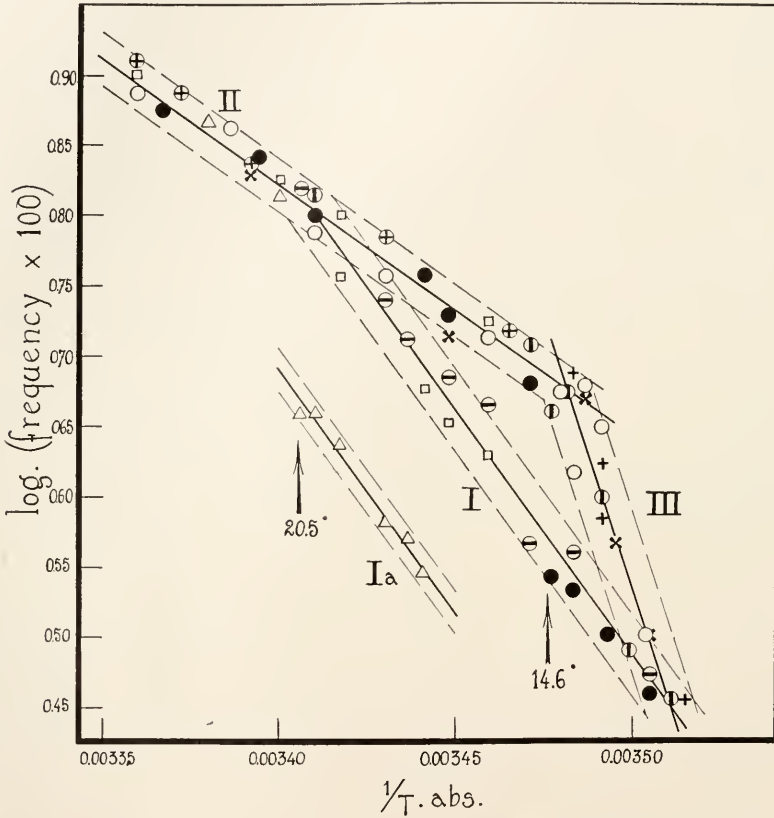

Fig. 1. Mass plot of all observations on *Mustelus canis*. Each animal has been represented by a different symbol. Each plotted point corresponds to the mean of 10 or 20 sets of readings, each reading being the time required for 20 gill movements. The number of sets of readings has been kept consistently 10 (or 20) for each animal; in other words, for any one animal there are 10 (or 20) sets of readings at each temperature.

26, p. 552) with the goldfish, after the animal was kept for 3 hours at 25° C. They agree with records obtained in many other instances, and are therefore an excellent argument in favor of the idea of different reactions playing definite rôles within definite limits of temperature. Occasionally one finds some observational point departing totally from the rest of the collection, and in many cases these data fall exactly in

the other band. These abrupt departures are especially frequent around critical temperatures, making these points very obvious in the graph when all observations are plotted separately. Necessarily all illegitimate averaging smooths out such points, which at the same time often lowers the absolute value of these average frequencies.

Changes of slopes of this nature are not surprising, as they have been found previously. They may perhaps indicate here that the " fundamental mechanism " determining the frequency of gill movement, which yields $\mu = 16{,}400$, may be in its turn linked with processes having lower (8,200) and higher (35,000) values of the critical increments. It has been suggested by Crozier and Federighi (1924–25) that the value $\mu = 8{,}200$ may be perhaps understood as reflecting the velocity of central nervous processes concerned in respiratory movements of fishes and other vertebrates. In all cases we may consider that the " fundamental mechanism " is by no means a simple one but must be probably of catenary type, more or less complicated, but where one of the three reactions (here characterized by their respective values of μ) plays the rôle of master reaction at any one moment.

We have not tried to find out any possible correlation existing between breathing and cardiac rhythms, as this would have obliged us to introduce in the animal pieces of apparatus which undoubtedly would have altered the breathing rhythm at least. We had noticed that the placing of a small glass rod (which has to be applied with utmost care in order to avoid all indirect stimulation) in the immediate vicinity of the opening of the spiracles affected singularly the coördination existing between spiracles and gill slits. Occasionally " beats " of the spiracles (in certain cases 3–4–5 in succession) are skipped although the gills operate at their normal constant rhythm. Scott (1913) had noticed also the relative independence of heart and gill movements, where he (p. 61) notes that the heart can continue to beat long after respiration has ceased, and that even when both organs are in action their rates may be increasing and decreasing independently of one another. On the other hand, Lyon (1926, p. 282) says that " even without artificial respiration, the heart may continue active long after breathing ceases " and on page 283 " that the heart rate of the sand shark is intimately related to the respiration rate. Usually the two are equal . . . the heart . . . normally takes its rate from respiration." One must remember that these experiments were performed with the sand shark (*Carcharias*), which has a slightly different type of water movement path than *Mustelus canis,* and that furthermore, as these animals were operated upon to introduce canulæ and connecting solutions for blood pressure determinations, and were kept partly out of water, they were

far from being in a state of " no interference " to which we wished to
restrict ourselves.

The respective positions of the bands in Fig. 1 determine at least
two critical temperatures: *I* and *II* intersect at about 20.5° C.; *II* and
III, at about 14.5° C. The fact that these temperatures occur as critical
temperatures in other respiratory activities (*cf., e.g.,* respiration of
Molge, Rana, Bufo for 15° C., Crozier, 1924, p. 198; respiration of
Vicia faba for 20.5° C., Navez, 1929, p. 656) is rather striking. Crozier
and Stier had noticed also that at temperatures above about 15° C.,
the pectoral fins of the goldfish were rather active, affecting by their
movements the breathing rhythm, and that below this temperature no
such disturbing activity was exhibited. We pointed out previously
that the latitude of variation of the data was rather small, of the order
of 1 to 2 per cent. Only in the vicinity of 14.5° C. do we get a varia-
tion which is, at its maximum, 7 per cent, but which, on both sides of
this temperature, reduces to its normal size. The fact that we did not
get such changes in the latitude of variation for the other critical tem-
perature seems to indicate that this variation is in itself indicative of
some definite change taking place in the organism, conceivably—for
example—modifications in the reactions controlling discharge in the
synapses. This would be consistent with the idea of assigning the
value $\mu = 8,000 \pm$ to processes of central nervous origin determining
breathing movements in the dogfish.

Another point may be made in relation to this matter of variation.
When we speak here of " variation," we mean the tendency of such
data to be scattered, in a seemingly random manner, in the band (Fig.
1) that we fit to them, and do not have reference to the error or mistake
that the observer could have introduced involuntarily. The variation
found in the data is *in* the material, truly a property of the organism,
and not due to some lack of precision in the measurements. How could
we in fact understand that the observer could make such errors that
would cause all his data, after averaging, taking of reciprocals and
logarithms, to fall in a band with parallel edges? A variation pre-
senting such properties ought to permit us to analyze it as a function
in itself. In other words we can consider, in the behavior of the organ-
ism, the behavior of a mathematical function and try to correlate one
with the other. Analogous cases have been described by Crozier (1929).

First, why are the bands with parallel edges? It might be objected
that it is impossible to conceive a regularity in the observations that
would give such bands. It may be urged that viscosity, and the general
complexity of protoplasm, would make the scatter of observational data
rather irregular. One may point out that it would be astonishing if

in the very large number of cases now on record, such things as viscosity would have behaved consistently in a way that paralleled the behavior of the process considered; in other words, that viscosity and other properties should have a series of temperature characteristics always the same as that of the process under study. Furthermore, would it not be strange that from worms up to mammals the rule governing viscosity, for instance, would be similar to the one controlling respiration or cardiac rhythm? Is it not rather a case where the very inconsistent appearance of the data of some authors is indicative, more of the ways of operation of the observer than of some fundamental character so easily ascribed to protoplasm? One *could* advance several reasons why divergences from a band with parallel edges " should " be found, but in the absence of such effects they need not be considered.
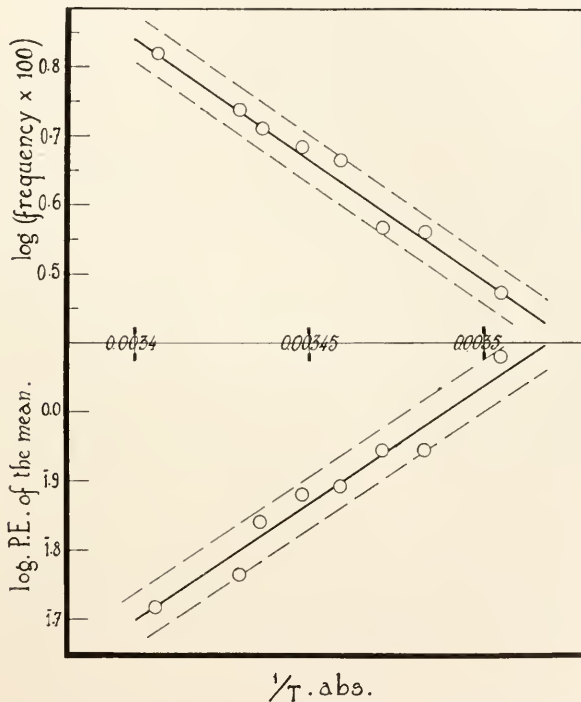

FIG. 2. The upper half of the graph is a reproduction of the points obtained for the animal represented by circles with horizontal diameters in Fig. 1. The lower half is a plot of the log of the P.E. of the mean frequencies of gill movements as a function of temperature. The slopes of the two lines are equal.

Let us go back to Fig. 1 and select out of the series of observations two sets to illustrate another point: a series (represented by a circle with an horizontal diameter) occurring in band *I*, and a second series

(indicated by solid circles) occurring in bands *I* and *II*. Each one of these series, plotted separately as in Figs. 2 and 3, shows this distribution of points in bands with parallel edges. We have represented also in the lower half of each figure the relation of the P.E. of the mean time for 20 beats to temperature for each of the points repre-
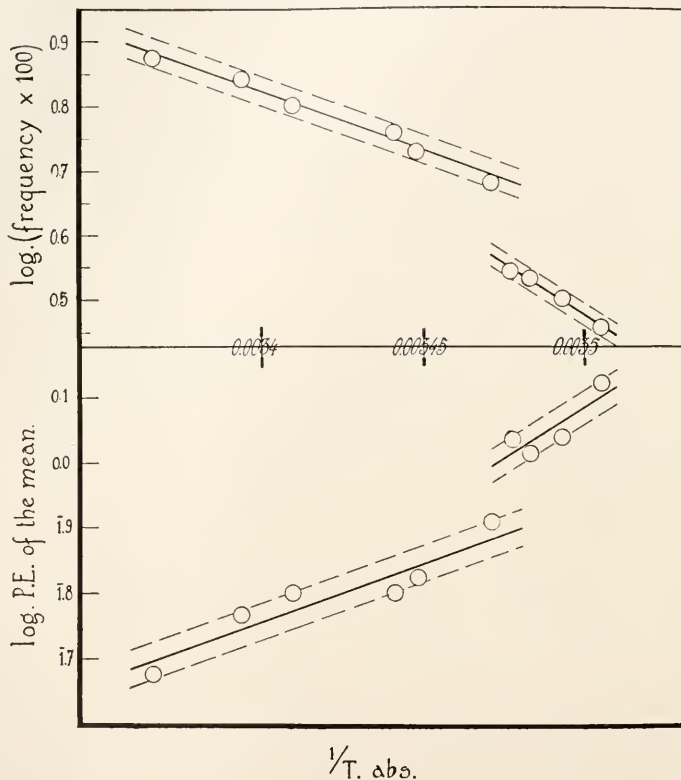

Fɪɢ. 3. The upper half of the figures is a reproduction of the points obtained for the animal represented by solid black circles in Fig. 1. The lower half is a plot of the log of the P.E. of the mean frequencies of gill movements as a function of temperature. The slopes of the lines are equal, and the break in each curve is located at the same temperature.

sented in the upper part. In each case the slope of the best-fitting line in the P.E. graph is the same as that obtained for the mean frequencies (*cf.* Crozier, 1929). In other words, the latitude of variation of the observations is a constant fraction of the mean, and both mean and variation of the mean are affected by temperature in the same way. Their temperature characteristics are the same. This is particularly illustrated in the second case where a definite shift from one value of

μ to another occurs.   The P.E. curve shows the same break at the same point on the thermometric scale.

   Two other conclusions can be drawn also: (1) a straight line relationship must exist between the P.E. of the mean (or the standard deviation of the mean) and the mean itself, (2) the standard deviation of the mean divided by the mean (*i.e.*, the coefficient of variability of the mean) must have no temperature characteristic (*i.e.*, the slope against $1/T$ must be zero.   Each one of these conclusions has been illustrated in Figs. 4 and 5.   [The use of σ_m (the standard deviation of the mean)
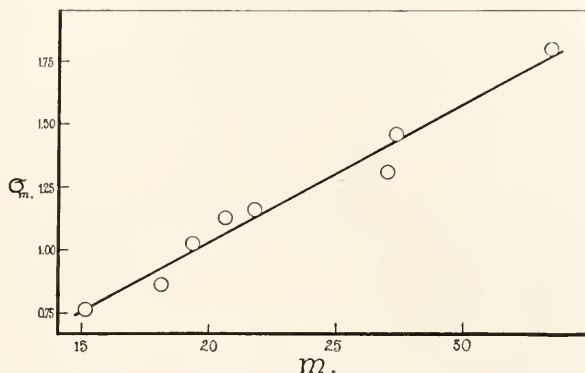

FIG. 4.   The standard deviation of the mean frequency of gill movements is a linear function of the mean.

instead of the P.E. affects only the size of the unit and not the form of relation.]   Obviously the straight line relationship is obeyed and the seemingly best-fitting line we draw through the scattered points of Fig. 5 is a line with a slope equal to zero or very close to it.
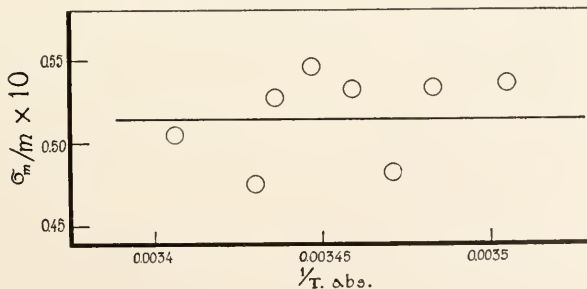

FIG. 5.   The "variability" of the mean (*i.e.*, the standard deviation of the mean divided by the mean) as a function of temperature; this "variability" is constant.

An analogous case has been described by Crozier (1929, p. 95) in relation to the behavior of guinea pigs on an inclined plane. The angles of orientation assumed by the animals on the plane for different inclinations of the plane show a very marked change in their variation at a given point (45°). The probable error plotted versus the angle of tilt of the plane shows a marked break at 45°, much more marked indeed than the one in the regular plot of orientation versus tilt of plane. This change in a mathematical function can, here also, be definitely correlated with reactions in the animal: the type of creeping is different above and below 45°.

These observations lead to the conclusion that in the cases where experiments have been made in a controlled way, where averaging of data has been done with discrimination, and where, as a whole, the observations are correspondingly weighted, we can study not only the phenomenon itself, but also the laws of variation of this phenomenon. These provide an additional check on the existence of critical temperatures and enable us to fix them in a quite independent way. In such cases, the study of the P.E. and of the coefficient of variability will confirm for us the idea that biological material may not be so " variable " as it is often taken to be.

Observations were made also on the spiny dogfish (*Squalus acanthias*). These data are identical with those in Fig. 1 giving the μ value of 16,400. Here also, in a few instances we found sudden shifts to the value 35,000. As the observations were not numerous enough, they are not given in detail.

We seem to have here a case homologous with the one found by Crozier (1924) in the data of Miss Leitch (1916) on the rate of development of the radicle of *Pisum*. Crozier showed (*cf.* 1924, Fig. 13, p. 207) that in this case these increments can be found: one of 16,450 when the observations were taken over periods of 0.5 hour; a second and a third one (over different temperature ranges) when the determinations were carried over periods of 22.5 hours. This last series of increments was obtained for data which included thus the hours of darkness and the daily rhythm of cell division in the plants. The former, on the contrary, were taken over short periods which could thus " average " out any possible trend due to the moment of observation with respect to the normal division rhythm.

The possible coexistence of such a series points to the fact that any one of two (or three) factors can act at any moment as " pace maker," impressing thus its temperature characteristic on the whole system. Our case is homologous in that we also have found for different animals the shift of one increment to another indicating a corresponding shift

in the pace makers of the system. Any one of these could come into control and last for a certain time, and then be replaced by another one determining another thermal increment for the system.

Summary

The temperature characteristic or critical thermal increments (μ) for the movement of the spiracles and gill slits in the dogfish *Mustelus canis* (and *Squalus acanthias*) are 8,200; 16,400 and 35,000 calories. Critical temperatures are found at 14.5° C. and at 20.5° C. The latitude of variation of the measurements varies in a definite way and this is correlated with the occurrence of a critical temperature of 14.5° C.

The reactions controlling breathing movements seem to be catenary processes, one of which may be concerned with the central nervous control of the breathing movements, over a certain range of temperatures.

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